ELSEVIER

Journal of Cereal Science ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jcs

Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from maize bran

Mirko Bunzel[a,*], Ella Allerdings[a], John Ralph[b,c], Hans Steinhart[a]

[a]*Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany*
[b]*US Dairy Forage Research Center, USDA-ARS, 1925 Linden Drive West, Madison, WI 53706-1108, USA*
[c]*Biological Systems Engineering Department, University of Wisconsin, Madison, WI 53706, USA*

Received 14 October 2006; received in revised form 22 December 2006; accepted 28 December 2006

Abstract

Dehydrodiferulates are likely the most important arabinoxylan cross-links in cereals and grasses in general. However, association of dehydrodiferulates and arabinoxylans has only been authenticated for 5-5- and 8-*O*-4-dehydrodiferulates to date. In the present study, a saccharide ester of 8-8(cyclic)-dehydrodiferulate was isolated from maize bran insoluble fibre following mild acidic hydrolysis by using Sephadex LH-20 chromatography, gel chromatography on Bio-Gel P-2, and RP-HPLC. Mass spectrometry, one- and two-dimensional NMR and analysis of the carbohydrate and phenolic constituents following further hydrolysis identified the isolated compound as the di-5-*O*-L-arabinosyl ester of 8-8(cyclic)-dehydrodiferulic acid. From this finding it is apparent that 8-8(cyclic)-dehydrodiferulate exists as such in the plant cell wall and acts as an arabinoxylan cross-link. In addition, a fraction was isolated that contained two saccharide esters of 8-*O*-4-dehydrodiferulates. This fraction was comprised of two compounds, both built from 8-*O*-4-dehydrodiferulate, a 5-linked arabinofuranose and a 5-linked xylopyranosyl-(1→2)-arabinofuranose unit. These compounds show that, in addition to the 5-*O*-(*trans*-feruloyl)-L-arabinofuranosyl sidechain, the more complex β-D-xylopyranosyl-(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranosyl sidechains are involved in the formation of 8-*O*-4-dehydrodiferulates.

Keywords: Diferulic acid; Ferulic acid; Cell-wall cross-linking; Arabinoxylans; Dietary fibre

1. Introduction

Cereal grain arabinoxylans are important dietary fibre compounds and they also influence formation and properties of dough and bread (Andersson and Aman, 2001). As cell wall constituents of grasses in general, arabinoxylans are also an important source of energy for ruminants (Hatfield et al., 1999). The physicochemical properties of arabinoxylans are dependent on the arabinose/xylose ratio, the distribution of the sidechains, the degree of polymerisation, the extent of their cross-linking, and on their coupling to other polymeric cell wall components. Arabinoxylan cross-linking and cross-coupling to other cell wall components such as lignin or proteins is achieved via hydroxycinnamates, especially via ferulate and its derivatives (Bunzel et al., 2004a; Geissmann and Neukom, 1971; Markwalder and Neukom, 1976; Piber and Koehler, 2005; Ralph et al., 1994, 2004). Ferulates acylate xylan arabinosyl units at their *O*-5-positions (Ishii, 1997).

Radical coupling of feruloylated arabinoxylans results in the formation of dehydrodiferulates or higher ferulate dehydrooligomers such as dehydrotriferulates and dehydrotetraferulates (Bunzel et al., 2003, 2005, 2006; Funk et al., 2005; Rouau et al., 2003). The ferulate moieties are coupled to produce new bonds that may occur at the 4-*O*-, 5- or 8-carbons, thus forming 5-5-, 8-8-, 8-5-, 8-*O*-4- and 4-*O*-5-linkages. Dehydrodiferulates are now routinely analysed in a whole range of plant materials following alkaline

Abbreviations: Ara, arabinose; DFA, dehydrodiferulic acid; TFA, trifluoroacetic acid; Xyl, xylose

*Corresponding author. Tel.: +49 40 42838 4379; fax: +49 40 42838 4342.
E-mail address: mirko.bunzel@uni-hamburg.de (M. Bunzel).

0733-5210/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2006.12.005

hydrolysis. Saponification, however, can modify the structures of the dehydrodiferulates involved. It is assumed that 5-5-, 8-*O*-4- and 4-*O*-5-dehydrodiferulic acids (DFA) are present as their ester analogs in the cell wall. This was also shown for 5-5- and 8-*O*-4-DFA by isolation and identification of 5-5- and 8-*O*-4-DFA-oligosaccharides following enzymatic or mild acidic hydrolysis (Allerdings et al., 2005; Ishii, 1991; Saulnier et al., 1999). From model reactions it is assumed that the 8-5(cyclic)-DFA is the natural form whereas the 8-5(open)- and 8-5(decarboxylated)-DFAs are saponification products (Ralph et al., 1994). The situation is less clear regarding the 8-8-coupled DFAs that are often the major dehydrodimers in cereal soluble fibers (Bunzel et al., 2001) and water-extractable rye arabinoxylans (Cyran et al., 2003). In addition to the formerly known dimers 8-8(cyclic)- and 8-8(open)-DFA, a third 8-8-coupled DFA, referred to as 8-8(tetrahydrofuran)-DFA, has been recently synthesised and identified in cereal grains (Schatz et al., 2006). Although we assume that all 8-8-coupled DFAs exist as such in the cell wall (Schatz et al., 2006), proving this assumption is of major interest. For example, studies to examine the ability of esterases to cleave various diferulate esters (Kroon, 2000; Wong, 2006) are only useful to the extent that model esters examined correspond to analogs present in the plant. The same is true concerning studies on the nutritional properties of ferulate dehydrodimers. 8-8-DFAs have been shown to be good antioxidants in copper-induced LDL oxidation test systems exceeding the activity of monomeric ferulate (Neudörffer et al., 2004). The 8-8(open)-dehydrodimer was shown to be more active than the cyclic counterpart also exhibiting different modes of action (Neudörffer et al., 2006). It is proposed that the structural integrity of wheat bran is increased by the presence of 8-8(cyclic)-DFA (Parker et al., 2005). Beyond the cereal grains, the 8-8(cyclic)-DFA is purported to play a key role in the thermal stability of Chinese water chestnut (Parker et al., 2003).

In this paper we describe the isolation and identification of a diarabinosyl 8-8(cyclic)-dehydrodiferulate from maize bran, proving that 8-8(cyclic)-dehydrodiferulates exist as such in the plant. In addition, we describe further 8-*O*-4-DFA-oligosaccharides implicating further xylosyl substitutions on the diferuloylated arabinose sidechains.

2. Experimental

2.1. Plant materials

Maize bran (*Zea mays* L.) was kindly provided by Hammermühle Maismühle GmbH (Kirrweiler, Germany).

2.2. General

Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1, from *Bacillus licheniformis*, 120 KNU/g) was from Novo Nordisk (Bagsvaerd, Denmark). Amberlite XAD-2 was obtained from Serva (Heidelberg, Germany), Bio-Gel P-2 from Bio-Rad Laboratories (Hercules, CA, USA), and Sephadex LH-20 was from Pharmacia Biotech (Freiburg, Germany). Analytical (Nucleosil 100-5 C18 HD, 250 × 4 mm, 5 µm) and semipreparative (Nucleosil 100-5 C18 HD, 250 × 10 mm, 5 µm) C18-HPLC columns were from Macherey-Nagel (Düren, Germany), and the analytical phenyl-hexyl-HPLC column (Luna Phenyl-Hexyl 100A, 250 × 4.6 mm i.d., 5 µm) was from Phenomenex (Aschaffenburg, Germany). GPC and HPLC equipment was from Merck-Hitachi (L-6000, L-6200 and L-7150 pumps, L-7400 UV detector equipped with a preparative flow cell; Darmstadt, Germany) and from Waters (994 programmable photodiode array detector; Eschborn, Germany). HPLC-MS was carried out on a Hewlett Packard system (HP Series 1100: autosampler G1313A, bin pump G1312A, degasser G1322A, mass spectrometer G1946A, ion-source: atmospheric pressure electro-spray ionisation, Hewlett Packard, Waldbronn, Germany). GC was performed on a Thermo Electron gaschromatograph (Focus GC, AI 3000 autoinjector, flame ionization detection (FID); Thermo Electron Corporation, Dreieich, Germany) using a J&W Scientific (Folsom, USA) DB-5 capillary column (0.32 mm × 30 m, 0.25 µm film thickness). 1D and 2D NMR-spectra were acquired on a Bruker DMX-750 instrument (Karlsruhe, Germany) fitted with a 5-mm triple-resonance (^{1}H, ^{13}C, ^{15}N) gradient inverse probe (proton coils closest to the sample).

2.3. Preparation of insoluble maize bran fibre

Maize bran (20 g, defatted with *n*-hexane, milled to a particle size smaller than 0.5 mm) was suspended in phosphate buffer (pH 6.0, 0.08 M, 300 ml), and heat-stable α-amylase (1.5 ml) was added. Beakers were placed in a boiling water bath for 40 min and shaken every 5 min. After cooling to room temperature, the suspension was centrifuged (2000 × *g*, 10 min), and the residue was washed twice with hot water (70 °C), 95% (v/v) ethanol, and acetone, and was finally dried at 40 °C overnight in a vacuum oven. The isolation procedure was performed nine times to produce about 80 g of insoluble fibre.

2.4. Acidic degradation of insoluble fibre

Mild acidic degradation of insoluble fibre was carried out as described previously (Bunzel et al., 2002; Saulnier et al., 1995) but with minor modifications. In brief, insoluble fibre (40 g) was treated with 50 mM trifluoroacetic acid (TFA, 1000 ml) under reflux for 3 h at 100 °C. After centrifugation the supernatant was filtered and evaporated at 40 °C under vacuum to 500 ml. The solution was adjusted to pH 5.0 using 0.1 M NaOH, centrifuged and filtered. This procedure was repeated and the solutions from both preparations were combined and evaporated under vacuum to 300 ml.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

2.5. Isolation of diarabinosyl 8-8(cyclic)-dehydrodiferulate (compound **1**, Fig. 1)

The hydrolysate was applied to a column (40 × 5 cm) of Amberlite XAD-2 and elution was carried out with H_2O (600 ml), MeOH/H_2O 50/50 (800 ml) and MeOH (350 ml). The MeOH/H_2O fraction was concentrated to 25.5 ml and applied to a column (100 × 3.2 cm) of Sephadex LH-20. Elution was carried out with water for 73 h (flow rate: 1 ml min^{-1}). Subsequently, all remaining compounds were rinsed from the column using methanol as eluent (flow rate: 1 ml min^{-1}). Elution was monitored continuously at 325 nm. The methanol eluate was concentrated to 25 ml and applied to a column (100 × 2.5 cm) of Bio-Gel P-2. Elution was carried out with water (flow rate: 0.5 ml min^{-1}) and monitored at 325 nm. Five major fractions were collected (Fig. 2a): F1, 31 mg; F2, 35 mg; F3, 21 mg; F4, 19 mg; F5, 12 mg. Fraction F2 was further separated using semipreparative C18-HPLC using the following gradient: eluent A, acetonitrile; eluent B, bi-distilled water; initially 10% A, 90% B, linear over 30 min to 30% A, 70% B, held isocratically for 10 min, followed by a rinsing and equilibration step. Elution was carried out at 40 °C and a flow rate of 2.5 ml min^{-1} was maintained. The injection volume was 50 ml using a 100 ml injection loop. Compound **1** eluted after 17.1 min. Isolation of 4.1 mg of compound **1** for structural analysis was achieved.

2.6. Isolation of 8-O-4-dehydrodiferulate saccharides (compounds **2a** and **2b**, Fig. 1)

Although compounds **2a**/**b** were also found in the Bio-Gel P-2 fraction F3 from the isolation procedure described in Section 2.5, the material used for structural analysis was obtained from a slightly different isolation procedure. The preparation of the insoluble fibre, the mild acidic hydrolysis and the Sephadex-LH 20 chromatography were similar to the procedures described in Sections 2.3–2.5, differing only in details. Bio-Gel P-2 chromatography, however, was carried out using another column size (50 × 2 cm). A chromatogram demonstrating this separation is shown in Fig. 2b. Fraction F′3 contained 8-*O*-4-dehydrodiferulate oligosaccharides **2a/b**. Compounds **2a/b** were finally separated using semipreparative HPLC using the conditions described in Section 2.5, only differing in the gradient used: eluent A, acetonitrile; eluent B, bi-distilled water; initially 10% A, 90% B, linear over 35 min to 20% A, 80% B, held isocratically for 7 min, followed by a rinsing and equilibration step. Compounds **2a/b** eluted together after 35 min. Isolation of 3.4 mg of compounds **2a/b** for structural analysis was achieved.

2.7. Structural elucidation of 8-8(cyclic)- and 8-O-4-dehydrodiferuloyl saccharides

Molecular masses of the isolated dehydrodiferuloylated oligosaccharides were determined by HPLC-MS working in the positive ion mode using a fragmentor voltage of 60 V. Fast elution was carried out using an analytical C18-column and a gradient of eluent A, ammonium formate buffer (pH 3), and eluent B, acetonitrile: initially 85% A, 15% B, held for 2 min, linear over 5 min to 50% A, 50% B, held isocratically for 5 min.The injection volume was 20 µl, the column temperature 40 °C, and the flow rate was maintained at 0.7 ml min^{-1}.

Phenolic compounds were determined by analytical phenyl-hexyl-HPLC after alkaline hydrolysis. The purified compounds (about 0.3 mg of each compound) were saponified with NaOH (1 M, 200 µl, degassed with N_2) by stirring for two hours in the dark at room temperature. The

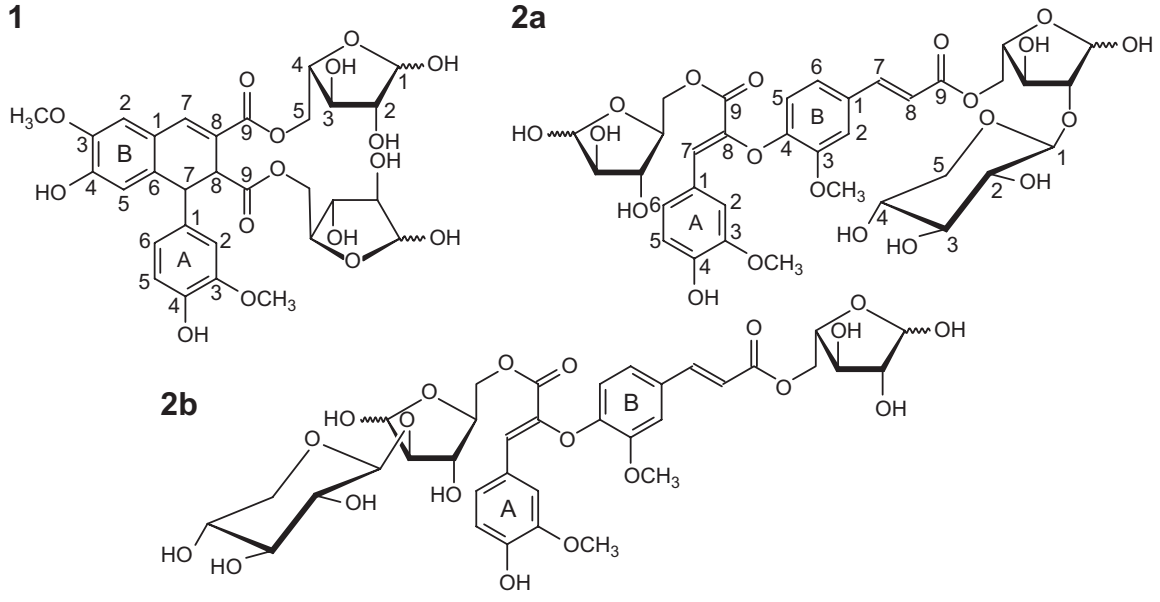

Fig. 1. Structures and numbering systems of isolated dehydrodiferuloyl saccharides 1, 2a and 2b.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

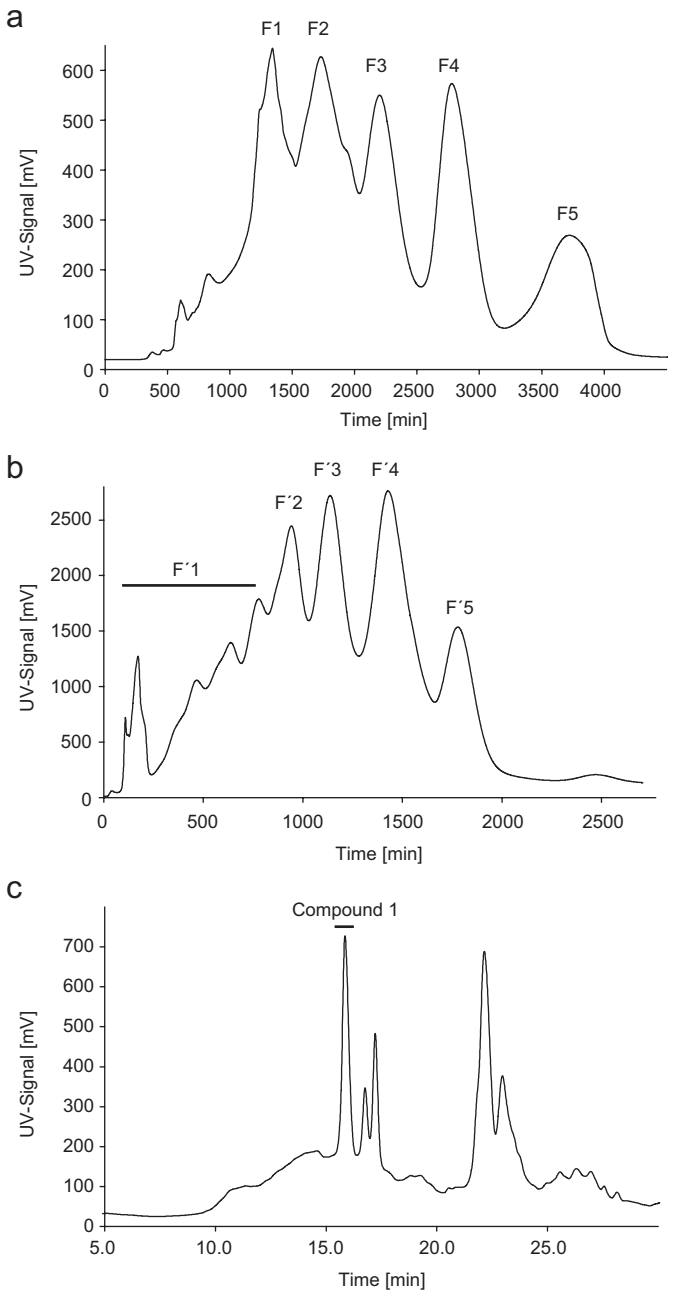

Fig. 2. Isolation of dehydrodiferuloyl saccharides. (a) Gel chromatography on Bio-Gel P-2 for the isolation of compound **1**. (b) Gel chromatography on Bio-Gel P-2 for the isolation of compounds **2a/b** using a column of different size. (c) Chromatogram of semipreparative RP-HPLC for the isolation of compound **1**.

reaction was stopped by adding 300 µl of a mixture of 2 M H_3PO_4 and MeOH (1/1, v/v) and the solution was used for HPLC analysis. The following gradient was applied to the separation of DFAs and ferulic acid: eluent A, 1 mM aqueous TFA; eluent B, acetonitrile; eluent C, MeOH/1 mM aqueous TFA (90/10, v/v); initially 85% A, 10% B, 5% C, held isocratically for 15 min, linear over 5 min to 75% A, 20% B, 5% C, linear over 20 min to 65% A, 25% B, 10% C, held isocratically for 10 min, linear over 5 min to 50% A, 30% B, 20% C, following rinsing and equilibration steps. The flow rate was 1 ml min^{-1} and the column temperature 45 °C. DFA standard compounds were isolated as described in Bunzel et al. (2004b).

Neutral carbohydrate compounds were determined as alditol acetates following acidic hydrolysis with 2 M TFA for 60 min at 120 °C, reduction and acetylation by GC-FID. Reduction and acetylation was carried out as described previously (Blakeney et al., 1983). GC conditions for the determination of the resulting alditolacetates were as follows: initial column temperature: 150 °C, held for 3 min, ramped at 5 °C min^{-1} to 200 °C, held for 1 min, ramped at 25 °C min^{-1} to 220 °C, held for 5 min, ramped at 20 °C min^{-1} to 300 °C; injector temperature 250 °C, detector temperature 300 °C. He (1.6 ml min^{-1}) was used as carrier gas.

For NMR analysis, compound **1** was dissolved in 0.5 ml of D_2O and acetone (0.5 µl) was used as reference (δ_H 2.22 ppm, δ_C 30.89 ppm) (Gottlieb et al., 1997). The mixture of compounds **2a/b** and the reference compound β-D-xylopyranosyl-(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranose were dissolved in 0.5 ml of 9:1 acetone-d_6:D_2O; the central acetone solvent peak was used as internal reference (δ_H 2.04 ppm, δ_C 29.80 ppm). Experiments used were standard Bruker implementations of gradient-selected versions of inverse (^{1}H-detected) homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC, HSQC-TOCSY), and heteronuclear multiple bond correlation (HMBC) experiments; the HMBC experiments used an 80 ms long-range coupling delay. Carbon shifts from compounds **1** and **2a**/**b** were taken from 2D HSQC, HSQC-TOCSY and HMBC experiments.

3. Results and discussion

3.1. Isolation of 8-8(cyclic)- and 8-O-4-dehydrodiferuloyl saccharides

Maize bran, which was used in former studies to isolate feruloyl and dehydrodiferulolyl saccharides (Allerdings et al., 2005, 2006; Saulnier et al., 1995, 1999; Saulnier and Thibault, 1999), was chosen as the plant material in this study. Due to its high amounts of ferulates and dehydrodiferulates (Bunzel et al., 2001; Saulnier and Thibault, 1999) maize bran is an ideal starting material to investigate polysaccharide cross-linking in cereal grains. Acidic hydrolysis of insoluble maize bran fibre, purification of the extract using Amberlite XAD-2 and Sephadex LH-20 chromatography are well proven methodologies for isolating the components of interest (Allerdings et al., 2005, 2006; Bunzel et al., 2002; Saulnier et al., 1995; Saulnier and Thibault, 1999). Sephadex LH-20 chromatography using water as eluent usually results in reproducible chromatographic separations. However, for the elution of dehydrodiferuloyl oligosaccharides organic modifiers such as ethanol or methanol have to be added to the aqueous eluent (Allerdings et al., 2005; Saulnier et al., 1999). As it turned out in recent experiments, the use of these eluents

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

led to chromatograms not being particularly reproducible. Therefore, we chose another strategy, eluting all dehydrodiferuloyl oligosaccharides together with pure methanol from the Sephadex LH-20-column and separating them by using chromatography on Bio-Gel P-2 with water as eluent (Figs. 2a and b), followed by semipreparative RP-HPLC (Fig. 2c). Although Bio-Gel chromatography is still not fully reproducible the chosen procedure seems to be more suitable than using Sephadex LH-20 chromatography with organic modifiers. Bio-Gel fractions (Fig. 2a) were roughly characterised using HPLC-DAD-MS. Characterisation was supported by proton and partially 2D-NMR data. From its UV-, mass- and NMR-spectra F5 was composed of a slightly contaminated compound, comprising 5-5-DFA and arabinoses, the di-5-*O*-L-arabinosyl ester of 5-5-DFA (Saulnier et al., 1999). F4 contained two major components comprised of a) 5-5-DFA, two arabinose units and one xylose unit and of b) 8-*O*-4-DFA and two arabinose units. These compounds were isolated and fully structurally characterized previously (Allerdings et al., 2005). F3 contained several compounds some of them being characterized following further separation to contain (a) 5-5-DFA, three pentoses and one hexose, (b) 5-5-DFA, four pentoses (from 2D-NMR very likely Xyl*p*(1→2)Ara*f*(5→)(5-5-DFA)(←5)Ara*f*(2←1)Xyl*p*)) and (c) 8-*O*-4-DFA and three pentoses (compounds **2a/b**), as fully characterized below. In addition to compound **1** (structural characterization detailed below), F2 contained several uncharacterized compounds, and F1 was characterized by a number of compounds built of a DFA and a single pentose. The composition of the isolated fractions showed that the separation on Bio-Gel P-2 does not strictly follow the rules of size exclusion chromatography, but that secondary interactions between the analytes and the stationary phase strongly influence the separation.

3.2. *Structural characterization of compound* **1**

The UV-spectrum of compound **1** was comparable to the UV-spectrum of 8-8(cyclic)-DFA, with the maximum at ~340 nm. From quasi-molecular ions in the positive ion mode ESI-MS spectrum (*m/z* 689 $[M+K]^+$ (base beak) and *m/z* 673 $[M+Na]^+$) the molecular weight of compound **1** was established to be 650, consistent with one

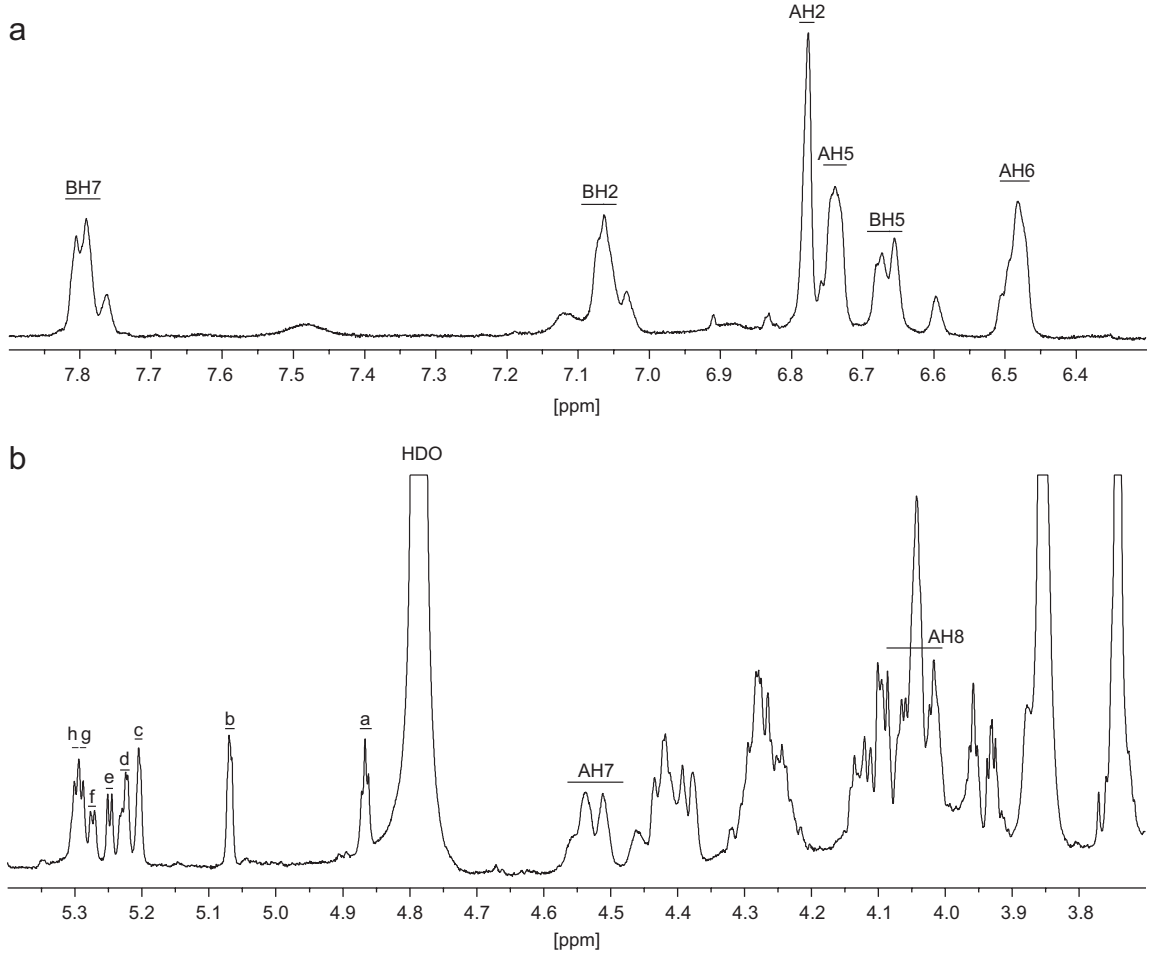

Fig. 3. ^{1}H-NMR of compound **1**. (a) Phenolic region. (b) Carbohydrate region. Note that the regions are differently scaled for better illustration. Proton signals from 8-8(cyclic)-dehydrodiferulate are labelled according to the numbering system shown in Fig. 1. Labels a–h represent arabinose anomeric proton signals as detailed in the text.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

dehydrodiferulate and two pentoses. Determination of the carbohydrates as their alditol acetates following acidic hydrolysis revealed arabinose to be the major sugar, only slightly (about 12%) contaminated with xylose. Phenolic compounds were identified and quantified by HPLC-DAD following alkaline hydrolysis at room temperature, establishing their esterified nature. The sole phenolic compounds identified were 8-8(cyclic)-DFA (~90%) and small amounts (~10%) of 5-5-DFA. From the analysis of the phenolic compounds and the carbohydrates the purity of compound **1** was estimated to be ~85–90%, contaminated by a 5-5-DFA-oligosaccharide. NMR of compound **1** was possible to acquire in D_2O contrary to reports of other DFA-oligosaccharides (including compounds **2a/2b**) that required acetone-*d*6/D_2O 9/1 (Allerdings et al., 2005) or CD_3OD/D_2O 1/1 (Saulnier et al., 1999).

As shown in Fig. 3a, the phenolic region of the proton spectrum is dominated by broad, less resolved signals. As detailed later, this is expected due to a range of possible isomers of compound **1**. Two proton signals characteristic for 8-8(cyclic)-DFA, AH7 and AH8, are located in the carbohydrate region of the proton spectrum at ~4.52 and ~4.04 ppm (Fig. 3b). Proton NMR data for the 8-8(cyclic)-dehydrodiferulate presented in Table 1 are reported from the main signals without reporting coupling constants. Carbon data obtained from 2D HSQC and HMBC spectra are presented in Table 2. Comparison of the NMR data with reference data from 8-8(cyclic)-DFA (Ralph et al., 1994) confirmed the presence of this diferulate in compound **1**.

The anomeric region of compound **1** revealed eight signals representing anomeric arabinose protons (Fig. 3b). From coupling constants and carbon data, four of these signals were assigned to stem from α-anomer protons (signals a–d, Tables 1 and 2) and four signals having their origin in β-anomer protons (signals e–h, Tables 1 and 2). Two of the signals (a and d) are further dispersed without being considered in this assignment. Assignment of proton signals to these anomeric signals led to eight sets of proton signals. Only four anomeric signals (two arabinose units with two anomers, α and β, each) were previously noted for a similar component, the diarabinosyl 8-*O*-4-dehydrodiferulate (Allerdings et al., 2005). The enhanced diversity of the anomeric signals in the proton spectrum of compound **1** may have its origin in the fact that the arabinose units are close to each other, connected via only four carbons originating from the DFA. Thus, the nature of the anomeric configuration of arabinose unit A may influence the proton signals in arabinose unit B and vice versa. The influence of the anomeric proton can be far reaching, e.g. dispersing proton signals of the 8-*O*-4-dehydrodiferulate in the already mentioned diarabinosyl 8-*O*-4-dehydrodiferulate (Allerdings et al., 2005). Another possibility would be through-space-interactions of the anomeric protons. However, simple modelling studies did not indicate any proximities that might produce proton shielding or deshielding. Dispersion of the anomeric signals is also possible due to the stereocenters at the 7- and 8-positions of DFA unit A. It was not possible to ultimately clarify the further dispersing of the anomeric proton signals. Especially the dispersion of the α-anomeric signals over a comparatively wide range remains a question not possible to answer at this point. From 2D NMR experiments the eight sets of proton signals were tentatively assigned to the

Table 1
Partial ^{1}H NMR data of compound **1** in D_2O

	Arabinose Moieties[a]								Arabinose-Ref. [b]	
	a (α)	b (α)	c (α)	d (α)	e (β)	f (β)	g (β)	h (β)	α	β
H1	4.869[c]	5.068	5.204	5.223[c]	5.249	5.274	5.291	5.298	5.29	5.32
($J_{1,2}$)	(*3.7*)	(*3.0*)	(*2.0*)	(*2.5*)	(*4.5*)	(*4.5*)	(*4.9*)	(*5.0*)	(–)	(*4.4*)
H2	3.931	3.957	4.036	4.043	4.022	4.049	4.095	4.098	4.07	4.12
H3	3.724	3.745	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H4	4.090	4.063	4.278	4.278	n.d.	n.d.	n.d	n.d.	n.d.	n.d.
H5	4.076	4.128	4.250	4.250	4.062	4.133	4.237	4.217	4.31	4.27
H5′	4.384	4.283	4.412	4.412	4.303	4.287(?)	4.386	4.423	4.45	4.46

	8-8(cyclic)-Dehydrodiferulate[d]										
	H2	H5	H6	H7	H8	OMe		H2	H5	H7	OMe
Unit A	6.777	6.739	6.482	4.524	4.036	3.742	Unit B	7.067	6.665	7.797	3.856

Chemical shifts δ_H are given in ppm, coupling constants *J* in Hz.
[a]Since an unambiguous assignment of the four α-anomeric and the four β-anomeric proton signals to the arabinose units A and B was not possible (see text), signals in the anomeric region are numbered a–h. α-Anomeric units are represented by a–d, whereas e–h represent β-anomeric units. Anomeric proton signals were used as starting points for signal assignments. Note that assignments of 3- and 4-protons are possible, but too ambiguous to be presented.
[b]Reference data (in D_2O) taken from arabinose as constituent of 5-*O*-*trans*-feruloyl-L-arabinofuranose (Bunzel et al., 2002).
[c]Further dispersed signals (see Fig. 3) not reported.
[d]Broad signals, no coupling constants were determined, partial further signal dispersal (see Fig. 3) not reported.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

Table 2
^{13}C NMR data of compound **1** in D_2O

	Arabinose Moieties[a]								Arabinose-Ref.[b]	
	a (α)	b (α)	c (α)	d (α)	e (β)	f (β)	g (β)	h (β)	α	β
C1	101.3	101.4	101.8	101.8	95.8	95.8	95.9	95.9	101.9	96.0
C2	81.6	81.5	81.8	81.8	76.4	76.5	76.5	76.5	81.9	76.6
C3	75.2	75.6	76.4	76.4	74.8	74.8 (?)	74.8	74.8	76.7	75.0
C4	80.1	80.3	81.1	81.1	79.0	79.0	79.2	79.2	81.4	79.3
C5	64.0	64.4	64.7	64.7	66.5	66.2	65.8	66.1	64.1	65.7

	8-8(cyclic)-Dehydrodiferulate[c]									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	OMe
Unit A	134.6[d]								174.9[d]	56.4
	134.8[d]	112.6	148.0	144.4	116.0	121.1	45.7	48.0	175.1[d]	
									175.4[d]	
Unit B	124.3	114.0	147.4	148.5	116.4	131.7	140.9	124.3	168.5	56.5

	8-8(cyclic)-Dehydrodiferulic Acid-Ref.[e]									
Unit A	136.1	112.0	148.1	146.1	115.5	120.7	46.0	48.1	173.6	56.2
Unit B	124.6	113.1	147.5	149.3	116.9	132.4	137.6	124.3	168.0	56.4

Chemical shifts δ_C are given in ppm.
[a]Since an unambiguous assignment of the four α-anomeric and the four β-anomeric proton signals to the arabinose units A and B was not possible (see text), signals in the anomeric region in Table 1 are numbered a–h. α-Anomeric units are represented by a–d, whereas e–h represent β-anomeric units. Carbon shifts shown here were assigned to these eight systems. Where no proton shifts were assigned in Table 1, carbon shifts were taken from the HSQC-TOCSY experiment and confirmed via data from the HMBC experiment; where proton shifts were assigned, carbon shifts were taken from the HSQC data and confirmed with data from the HSQC-TOCSY and HMBC experiments.
[b]Reference data (in D_2O) taken from arabinose as a constituent of 5-*O*-*trans*-feruloyl-L-arabinofuranose (Bunzel et al., 2002).
[c]Carbon shifts taken from HSQC where possible and for quaternary carbons from HMBC data.
[d]Carbon signals were dispersed in the HMBC. Other signals are partially also dispersed in the HMBC spectrum, but usually not by more than 0.1 ppm. Exceptions are the carbon shifts for carbon A7 (dispersed into signals between δ_C 45.6 and 46.3 ppm) and carbon A8 (dispersed into signals between δ_C 47.9 and 48.3 ppm).
[e]Reference data (in acetone-d_6) taken from Ralph et al. (1994).

arabinose units A and B. The key for this classification is the marked difference in the carbon-9 shifts of the DFA units A and B. Long-range couplings via three bonds correlate C9 with the 5-protons of the arabinose units. From this information the proton sets a, b, e and f were assigned to arabinose unit A, whereas the proton sets c, d, g and h presumably belong to arabinose unit B. However, this assignment is not unambiguous.

Arabinose carbon data were deduced from 2D HSQC and HSQC-TOCSY experiments, and confirmed via the HMBC experiment; the HSQC-TOCSY experiment (Fig. 4) was especially valuable where proton shifts were not unambiguously assigned. The carbon data are more consistent than the proton data, apparently representing α- and β-arabinofuranoses, as shown in Table 2 by comparison to arabinose carbon shifts from 5-*O*-*trans*-feruloyl-L-arabinofuranose. The HMBC-spectrum (Fig. 5) clearly showed the ester linkages of the 8-8(cyclic)-DFA to the 5-positions of the arabinose units, and also verified the ring sizes of the arabinoses to be furanoses (not shown).

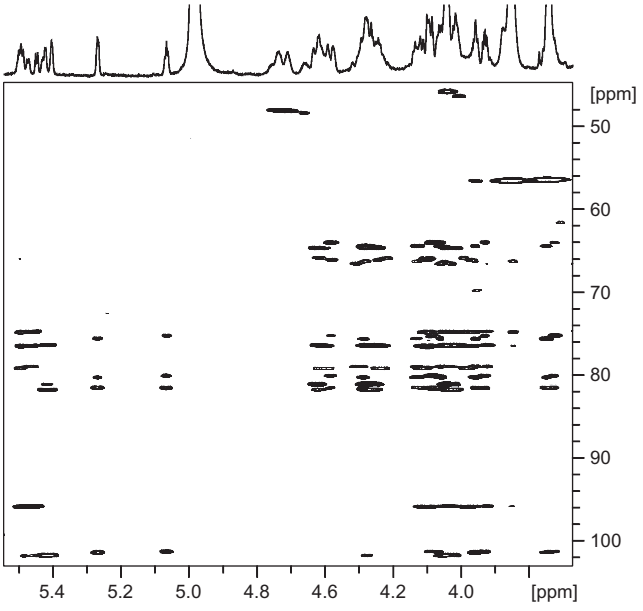

Fig. 4. HSQC-TOCSY spectrum of compound **1**, a valuable experiment in the assignment of carbon shifts.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

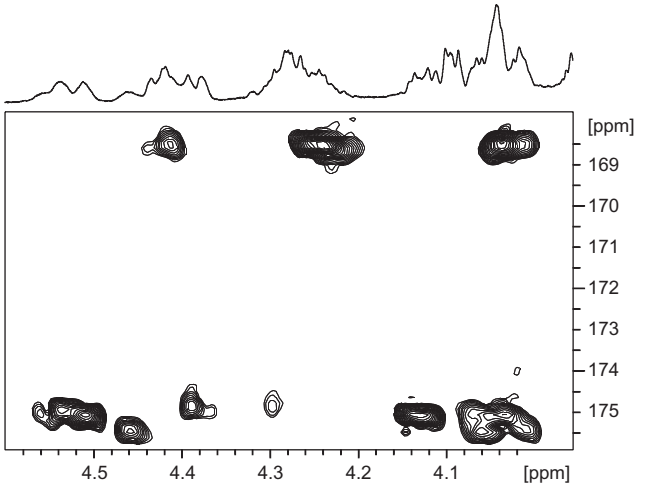

Fig. 5. Slice of the HMBC spectrum of compound **1** showing three bond correlations between 9-carbons of 8-8(cyclic)-dehydrodiferulate and 5-protons of the arabinose units.

3.3. Structural characterization of compounds **2a/2b**

The fraction containing compounds **2a/2b** had a UV-spectrum characteristic of 8-*O*-4-DFA with a broad shoulder between about 290 and 310 nm. From quasi molecular ions in the MS with *m/z* 821 $[M+K]^+$ (base peak), *m/z* 805 $[M+Na]^+$ and *m/z* 800 $[M+NH_4]^+$, the molecular mass was determined to be 782, corresponding to one DFA and three pentoses. These findings were confirmed and augmented from the results of the carbohydrate and phenolic acid analysis. Carbohydrate determination revealed an arabinose/xylose ratio of 2:1, with no other contaminating sugars at significant levels. 8-*O*-4-DFA was essentially the sole phenolic compound determined by HPLC-DAD following alkaline hydrolysis.

NMR data finally revealed that the isolated fraction contained both compounds **2a** and **2b** (Fig. 1). Each compound is comprised of 8-*O*-4-dehydrodiferulate, 5-linked arabinofuranose and the 5-linked disaccharide

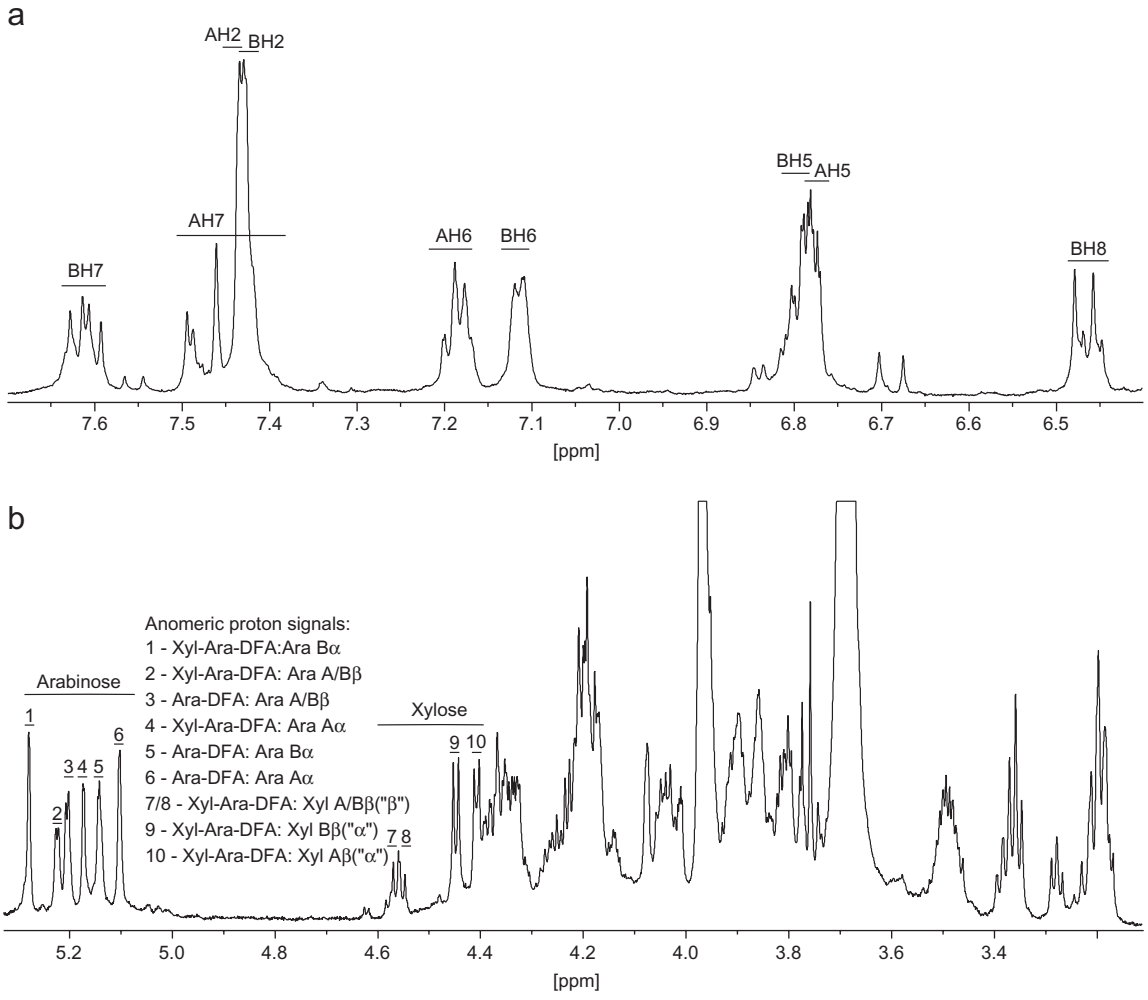

Fig. 6. ^{1}H-NMR of a mixture of compounds **2a/2b**. (a) Phenolic region. (b) Carbohydrate region. Note that the regions are differently scaled for better illustration. Proton signals from 8-*O*-4-dehydrodiferulate are labelled according to the numbering system shown in Fig. 1. 1–10 represent arabinose or xylose anomeric proton signals as described in the Figure. Xyl-Ara-DFA: Xyl(1→2)Ara(5→)diferulate; Ara-DFA: Ara(5→)diferulate; see also Table 3.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

xylopyranosyl-(1→2)-arabinofuranose. However, they differ in the arrangement of the carbohydrate substituents on the dehydrodiferulate moieties A and B. The NMR spectra of this mixture of two compounds, each of them being represented by four isomers having their origin in the two anomers of each of the arabinose units, are complex (Fig. 6). Although many details were deduced from these spectra (Tables 3 and 4) it was not possible to assign all signals. It was possible to follow signals that stem from α-arabinose units and it was also possible to assign the 5-linked α-arabinofuranoses and the 5-linked β-xylopyranosyl-(1→2)-α-arabinofuranoses to the A and B rings of the diferulate unit. However, this was not feasible for the 5-linked β-arabinofuranoses and the 5-linked β-xylopyranosyl-(1→2)-β-arabinofuranoses. For these carbohydrate constituents no assignments to the rings A and B were made because it was not possible to define two distinct sets of NMR signals. Therefore only the main signals in the 2D-experiments are given in Tables 1 and 2. The signals from the xylopyranose units that are primarily β-anomers are further split depending on the configuration of the adjacent arabinose unit.

Although not all signals were assigned, NMR data unambiguously identified compounds **2a** and **2b** as also shown by the comparison of the determined data with data from the reference compounds β-D-xylopyranosyl-(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranose and the di-5-*O*-L-arabinosyl ester of 8-*O*-4-DFA. The ester linkage to the 5-position of the arabinose units, the (1→2)-linkages between the xylose and arabinose units, and the ring size of the arabinose (furanose) and the xylose (pyranose) were confirmed by the HMBC experiment (Fig. 7).

Table 3
^{1}H NMR data of compounds **2a**/**b** in acetone-d_6/D_2O 9/1

	Xyl(1→2)Ara(5→)diferulate						Xyl (1→2)Ara (5→)ferulate-Ref.[a]			
	Arabinose[b]			Xylose			Arabinose		Xylose	
	Aα	Bα	A/Bβ[c]	Aβ ("α")	Bβ ("α")	A/Bβ ("β")[c]	α	β	β ("α")	β ("β")
H1	5.172	5.279	5.224	4.407	4.448	4.552 (*7.4*)	5.285	5.229	4.454	4.570
($J_{1,2}$)	(*1.6*)	(*1.1*)	(*3.7*)	(*7.5*)	(*7.6*)	4.565 (*7.6*)	(—)	(*3.9*)	(*7.6*)	(*7.4*)
H2	4.009	4.076	4.034	3.198	3.184	3.278	4.081	4.045	3.209	3.289
H3	3.947	4.051	4.264	3.356	3.358	3.379	4.047	4.271	3.380	3.390
H4	4.140	4.200	3.909	3.485	3.485	3.495	4.212	3.926	3.489	3.511
H5	4.200	4.180	4.248	3.178	3.178	3.206	4.164	4.177	3.204	3.222
H5′	4.399	4.368	4.346	3.806	3.806	3.842	4.365	4.336	3.814	3.843

	Ara (5→)diferulate[b]			Ara (5→)diferulate-Ref.[d]					8-*O*-4-DFA[e]		8-*O*-4-DFA-Ref.[d]	
	Aα	Bα	A/Bβ[c]	Aα	Bα	Aβ	Bβ		A	B	A	B
H1	5.103	5.143	5.204	5.102	5.135	5.197	5.202	H2	7.432	7.424	~7.43	~7.43
($J_{1,2}$)	(*1.5*)	(*2.3*)	(*3.9*)	(*2.1*)	(*2.3*)	(*4.5*)	(*4.1*)					~*7.41*
H2	3.956	3.976	3.906	3.956	3.972	3.911	3.907	H5	6.778	6.795	~6.77	~6.79
								H6	7.180	7.111	~7.17	~7.11
H3	3.857	3.891	4.030	3.862	3.886	4.030	4.028	H7	7.490	7.618	~7.50	~7.61
									7.458	7.604	~7.46	
H4	4.160	n.d.	3.863	4.169	4.192	3.883	3.866		7.423			
								($J_{7,8}$)		(*16.0*)		(*16.0*)
H5	4.220	n.d.	4.180	4.220	4.174	4.177	4.236	H8		6.469		~6.46
										6.459		~6.45
H5′	4.356	4.332	4.328	4.362	4.330	4.314	4.333	OMe	3.678	3.968	~3.67	~3.96

A or B describes the ferulate unit within the 8-*O*-4-diferulate (Fig. 1), α or β the arabinose/xylose configuration and "α" or "β" describes the influence of the arabinose configuration on the xylose signals. Chemical shifts δ_H are given in ppm, coupling constants *J* in Hz.

[a]Reference data from β-D-xylopyranosyl-(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranose in acetone-d_6/D_2O 9/1.

[b]Where proton data could not be assigned from the (H,H)-COSY experiment they were taken from the HSQC-TOCSY or from the HMBC spectrum. Although data were confirmed using all experiments that were carried out, interchanging of some signals, especially signals for 5-protons is not fully excluded.

[c]NMR signals from arabinose β-anomers were not possible to classify according to ring A or B—the given signals represent the main signals in 2D-spectra, especially in the (H,H)-COSY and HSQC-TOCSY experiments.

[d]Reference data (in acetone-d_6/D_2O 9/1) taken from the di-5-*O*-L-arabinosyl ester of 8-*O*-4-dehydrodiferulic acid (Allerdings et al., 2006); DFA—dehydrodiferulate.

[e]Due to complex signals, the centers only are reported. Only in case of distinct separation of resonances multiple values are given. Unambiguous reporting of coupling constants was only possible for the coupling of protons 7 and 8.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

Table 4
^{13}C NMR data of compounds **2a/b** in acetone-d_6/D_2O 9/1

	Xyl(1→2)Ara(5→)diferulate[a]						Xyl(1→2)Ara(5→)ferulate-Ref.[b]			
	Arabinose			Xylose			Arabinose		Xylose	
	Aα	Bα	A/Bβ[c]	Aβ("α")	Bβ("α")	A/Bβ("β")	α	β	β("α")	β("β")
C1	101.39	101.63	96.54	104.06	103.83	103.76	101.64	96.54	103.93	103.79
C2	91.17	90.95	84.25	74.05	74.05	73.70	91.07	84.37	74.12	73.79
C3	76.50	76.98	74.42 74.80	77.08	77.08	76.46	77.08!	74.84	77.09!	76.52
C4	80.09	80.57	79.86 79.44	70.18	70.18	70.18	80.67	79.86	70.30	70.23
C5	65.03	64.67	66.96	66.21	66.21	66.01	64.68	66.69	66.26	66.08

	Ara(5→)diferulate[a]			Ara(5→)diferulate-Ref.[d]				8-*O*-4-DFA[e]		8-*O*-4-DFA-Ref.[d]	
	Aα	Bα	A/Bβ[c]	Aα	Bα	Aβ	Bβ		A	B	A	B
C1	103.11	103.11	96.90	103.09	103.09	96.93	96.93	C1	124.70	129.83	~124.7	~129.8
								C2	113.82	112.26	~113.9	~112.2
C2	82.85	82.96	77.66	82.91	83.00	77.70	77.70	C3	148.26	149.87	~148.3	~149.9
								C4	149.50	148.53	~149.5	~148.6
C3	77.92	78.15	76.40	78.00	78.18	76.50	76.50	C5	115.90	114.27	~115.9	~114.3
								C6	126.04	123.05	~126.0	~123.0
C4	81.48	81.50	80.14	81.56	81.56	80.30	80.30	C7	~129.0[f]	145.56	~129.0	~145.5
								C8	137.34	116.79	~137.3	~116.8
								C9	163.74	167.59	~163.8	~167.7
C5	65.67	65.13	66.49	65.70	65.16	66.60	67.00	OMe	55.63	56.34	~55.7	~56.4

Chemical shifts δ_C are given in ppm. A or B describes the ferulate unit within the 8-*O*-4-diferulate (Fig. 1), α or β the arabinose/xylose configuration and "α" or "β" describes the influence of the arabinose configuration on the xylose signals.

[a]Carbon shifts taken from the HSQC-experiment.

[b]Reference data from β-D-xylopyranosyl-(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranose in acetone-d_6/D_2O 9/1.

[c]NMR signals from arabinose β-anomers were not possible to classify according to ring A or B—the given signals represent the main signals in 2D-experiments.

[d]Reference data (in acetone-d_6/D_2O 9/1) taken from the di-5-*O*-L-arabinosyl ester of 8-*O*-4-dehydrodiferulic acid (Allerdings et al., 2006); DFA—dehydrodiferulate.

[e]Carbon shifts taken from HSQC where possible and for quaternary carbons from HMBC data.

[f]Given value represents the centre of three signals at 129.14, 129.03 and 128.97.

3.4. Implications for arabinoxylan cross-linking

Isolation and identification of compound **1**, the diarabinosyl 8-8(cyclic)-dehydrodiferulate, proves the association of arabinoxylans and the 8-8(cyclic)-dehydrodiferulate. To date, this evidence has only been supplied for 5-5- and 8-*O*-4-dehydrodiferulates (Allerdings et al., 2005; Saulnier et al., 1999) but neither for 8-8-coupled dehydrodiferulates nor for the 8-5-dehydrodiferulate. In case of the latter one this is especially surprising since the 8-5-coupled dehydrodimer is the dominant alkali-releasable dehydrodiferulate in maize and other cereal grains (Bunzel et al., 2001). In addition to the observation that 8-8-coupled dimers indeed act as inter/intramolecular polysaccharide cross-links, our results also imply that 8-8(cyclic)-dehydrodiferulate exists as such in the plant cell wall and that it is not exclusively formed from an unknown precursor or, for example, from 8-8(tetrahydrofuran)-diferulate during saponification (Schatz et al., 2006). The only unknown in this premise is the behaviour of a possible unknown component during the conditions of the mild acidic hydrolysis. If we want to exclude this possibility, isolation of 8-8(cyclic)-DFA-oligosaccharides is necessary from an enzymatic hydrolysate. However, knowing from prior attempts the problems in the isolation process of diferuloylated oligosaccharides, such proof will not be readily forthcoming.

The isolation of compounds **2a** and **2b** shows also that more complex feruloylated sidechains and not only the monomeric arabinose sidechain are involved in arabinoxylan cross-linking. An additional xylose unit is obviously not a steric hindrance for the coupling of ferulate radicals as already shown for the formation of the 5-5-dehydrodiferulate by isolation and identification of a similar compound to **2a/b** but with 5-5-dehydrodiferulate as the phenolic compound (Saulnier et al., 1999). The finding of a 5-5-dehydrodiferulate coupled to three pentoses and one hexose in the present study is a first indication that also the α-L-galactopyranosyl-(1→2)-β-D-xylopyranosyl-

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

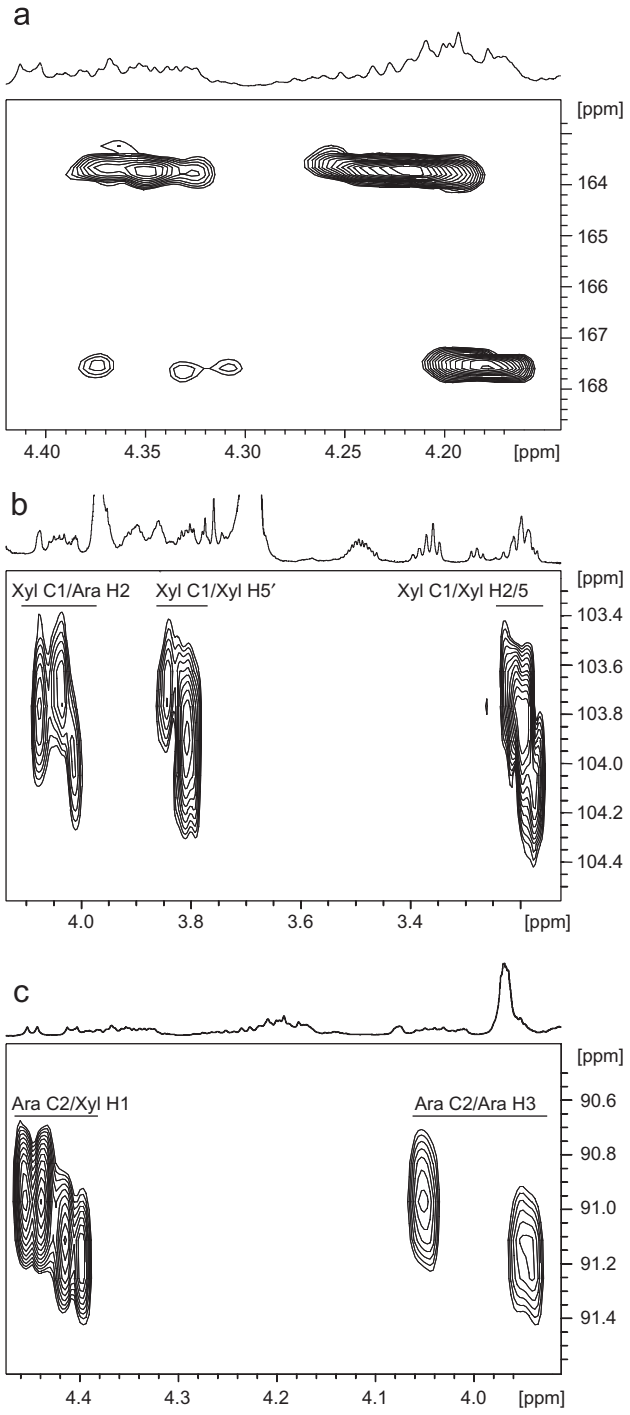

Fig. 7. Slices of the HMBC spectrum of the mixture of compounds **2a/b**. (A) Signals from three bond correlations between 9-carbons of 8-*O*-4-dehydrodiferulate and 5-protons of the proton signals. (B) and (C) Three bond correlations that prove the (1→2)-linkage between xylose and arabinose (Xyl C1/Ara H2, Ara C2/ Xyl H1) and establish the pyranose form of xylose (Xyl C1/Xyl H5,5′).

(1→2)-5-*O*-*trans*-feruloyl-L-arabinofuranose side chains (Allerdings et al., 2006) may be involved in forming arabinoxylan cross-links. However, due to some impurities it was not possible to obtain unambiguous NMR data for this compound.

References

Allerdings, E., Ralph, J., Schatz, P.F., Gniechwitz, D., Steinhart, H., Bunzel, M., 2005. Isolation and structural identification of diarabinosyl 8-*O*-4-dehydrodiferulate from maize bran insoluble fibre. Phytochemistry 66, 113–124.

Allerdings, E., Ralph, J., Steinhart, H., Bunzel, M., 2006. Isolation and structural identification of complex feruloylated heteroxylan side-chains from maize bran. Phytochemistry 67, 1276–1286.

Andersson, R., Aman, P., 2001. Cereal arabinoxylan: occurrence, structure, and properties. In: McCleary, B.V., Prosky, L. (Eds.), Advanced Dietary Fibre Technology. Blackwell Science Ltd., Oxford, pp. 301–314.

Blakeney, A.B., Harris, P.J., Henry, R.J., Stone, B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydrate Research 113, 291–299.

Bunzel, M., Ralph, J., Marita, J.M., Hatfield, R.D., Steinhart, H., 2001. Diferulates as structural components in soluble and insoluble cereal dietary fibre. Journal of the Science of Food and Agriculture 81, 653–660.

Bunzel, M., Allerdings, E., Sinnwell, V., Ralph, J., Steinhart, H., 2002. Cell wall hydroxycinnamates in wild rice (*Zizania aquatica* L.) insoluble dietary fibre. European Food Research and Technology 214, 482–488.

Bunzel, M., Ralph, J., Funk, C., Steinhart, H., 2003. Isolation and identification of a ferulic acid dehydrotrimer from saponified maize bran insoluble fiber. European Food Research and Technology 217, 128–133.

Bunzel, M., Ralph, J., Lu, F., Hatfield, R.D., Steinhart, H., 2004a. Lignins and ferulate-coniferyl alcohol cross-coupling products in cereal grains. Journal of Agricultural and Food Chemistry 52, 6496–6502.

Bunzel, M., Funk, C., Steinhart, H., 2004b. Semipreparative isolation of dehydrodiferulic and dehydrotriferulic acids as standard substances from maize bran. Journal of Separation Science 27, 1080–1086.

Bunzel, M., Ralph, J., Funk, C., Steinhart, H., 2005. Structural elucidation of new ferulic acid-containing phenolic dimers and trimers isolated from maize bran. Tetrahedron Letters 46, 5845–5850.

Bunzel, M., Ralph, J., Brüning, P., Steinhart, H., 2006. Structural identification of dehydrotriferulic and dehydrotetraferulic acids isolated from insoluble maize bran fiber. Journal of Agricultural and Food Chemistry 54, 6409–6418.

Cyran, M., Courtin, C.M., Delcour, J.A., 2003. Structural features of arabinoxylans extracted with water at different temperatures from two rye flours of diverse breadmaking quality. Journal of Agricultural and Food Chemistry 51, 4404–4416.

Funk, C., Ralph, J., Steinhart, H., Bunzel, M., 2005. Isolation and structural characterisation of 8-*O*-4/8-*O*-4- and 8-8/8-*O*-4-coupled dehydrotriferulic acids from maize bran. Phytochemistry 66, 363–371.

Geissmann, T., Neukom, H., 1971. Vernetzung von Phenolcarbonsäureestern von Polysacchariden durch oxydative phenolische Kupplung. Helvetica Chimica Acta 54, 1108–1112.

Gottlieb, H.E., Kotlyar, V., Nudelman, A., 1997. NMR chemical shifts of common laboratory solvents as trace impurities. Journal of Organic Chemistry 62, 7512–7515.

Hatfield, R.D., Ralph, J., Grabber, J.H., 1999. Cell wall structural foundations: Molecular basis for improving forage digestibilities. Crop Science 39, 27–37.

Ishii, T., 1991. Isolation and characterization of a diferuloyl arabinoxylan hexasaccharide from bamboo shoot cell-walls. Carbohydrate Research 219, 15–22.

Ishii, T., 1997. Structure and functions of feruloylated polysaccharides. Plant Science 127, 111–127.

Kroon, P.A., 2000. What role for feruloyl esterases today? Polyphénols Actualités 19, 4–5.

Markwalder, H.U., Neukom, H., 1976. Diferulic acid as a possible crosslink in hemicelluloses from wheat germ. Phytochemistry 15, 836–837.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005

Neudörffer, A., Bonnefont-Rousselot, D., Legrand, A., Fleury, M.-B., Largeron, M., 2004. 4-Hydroxycinnamic ethyl ester derivatives and related dehydrodimers: Relationship between oxidation potential and protective effects against oxidation of low-density lipoproteins. Journal of Agricultural and Food Chemistry 52, 2084–2091.

Neudörffer, A., Desvergne, J.-P., Bonnefont-Rousselot, D., Legrand, A., Fleury, M.-B., Largeron, M., 2006. Protective effects of 4-hydroxycinnamic ethyl ester derivatives and related dehydrodimers against oxidation of LDL: Radical scavengers or metal chelators? Journal of Agricultural and Food Chemistry 54, 1898–1905.

Parker, C.C., Parker, M.L., Smith, A.C., Waldron, K.W., 2003. Thermal stability of texture in Chinese water chestnut may be dependent on 8,8'-diferulic acid (aryltetralyn form). Journal of Agricultural and Food Chemistry 51, 2034–2039.

Parker, M.L., Ng, A., Waldron, K.W., 2005. The phenolic acid and polysaccharide composition of cell walls of bran layers of mature wheat (*Triticum aestivum* L. cv. Avalon) grains. Journal of the Science of Food and Agriculture 85, 2539–2547.

Piber, M., Koehler, P., 2005. Identification of dehydro-ferulic acid-tyrosine in rye and wheat: Evidence for a covalent cross-link between arabinoxylans and proteins. Journal of Agricultural and Food Chemistry 53, 5276–5284.

Ralph, J., Quideau, S., Grabber, J.H., Hatfield, R.D., 1994. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell-walls. Journal of the Chemical Society, Perkin Transaction 1, 3485–3498.

Ralph, J., Bunzel, M., Marita, J.M., Hatfield, R.D., Lu, F., Kim, H., Schatz, P.F., Grabber, J.H., Steinhart, H., 2004. Peroxidase-dependent cross-linking reactions of *p*-hydroxycinnamates in plant cell walls. Phytochemistry Reviews 3, 79–96.

Rouau, X., Cheynier, V., Surget, A., Gloux, D., Barron, C., Meudec, E., Louis-Montero, J., Criton, M., 2003. A dehydrotrimer of ferulic acid from maize bran. Phytochemistry 63, 899–903.

Saulnier, L., Vigouroux, J., Thibault, J.F., 1995. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. Carbohydrate Research 272, 241–253.

Saulnier, L., Crepeau, M.J., Lahaye, M., Thibault, J.F., Garcia-Conesa, M.T., Kroon, P.A., Williamson, G., 1999. Isolation and structural determination of two 5,5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. Carbohydrate Research 320, 82–92.

Saulnier, L., Thibault, J.F., 1999. Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans. Journal of the Science of Food and Agriculture 79, 396–402.

Schatz, P.F., Ralph, J., Lu, F., Guzei, I.A., Bunzel, M., 2006. Synthesis and identification of 2,5-di(4-hydroxy-3-methoxyphenyl)-tetrahydrofuran-3,4-dicarboxylic acid, an unanticipated ferulate 8-8-coupling product acylating cereal plant cell walls. Organic and Biomolecular Chemistry 4, 2801–2806.

Wong, D.W.S., 2006. Feruloyl esterase: a key enzyme in biomass degradation. Applied Biochemistry and Biotechnology 133, 87–112.

Please cite this article as: Bunzel, M., et al., Cross-linking of arabinoxylans via 8-8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8-8(cyclic)-dehydrodiferulate from..., Journal of Cereal Science (2007), doi:10.1016/j.jcs.2006.12.005